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First finds of "alder-Phytophthora" in the Czech Republic

KAREL ČERNÝ, BOŽENA GREGOROVÁ, VLADIMÍR HOLUB, VERONIKA STRNADOVÁ

Agency for Nature Conservation and Landscape Protection of the Czech Republic, Kališnická 4–6, Praha 3, 130 23 Czech Republic
cerny@nature.cz

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The new hybride "alder-*Phytophthora*" (*P. cambivora* × *P.* cf. *fragariae*) has originated in western Europe and its area has an expanding tendency. This pathogenic fungus was isolated during studies of declining alders in the river basin of the Ohře river at Chodovský potok brook near Karlovy Vary, western Bohemia. The fungus was found in a substrate with damaged roots and in conductive tissues of trunks of declining trees of *Alnus glutinosa*.

Key words: *Phytophthora*, *Alnus glutinosa*, alder decline

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Nový hybrid, tzv. „alder-*Phytophthora*" (*P. cambivora* × *P.* cf. *fragariae*), vznikl v západní Evropě a jeho areál se neustále rozšiřuje. Patogen byl izolován během studií chřadnoucích olší v povodí Ohře na Chodovském potoce poblíž Karlových Varů. Houba byla zjištěna v substrátu s poškozenými kořeny a ve vodivých pletivech hynoucích jedinců *Alnus glutinosa*.

INTRODUCTION

In the last decade a massive decline of alder (*Alnus glutinosa*, *A. incana*, and *A. cordata*) has been reported in western and central Europe (e.g. Gibbs et al. 1999). Declining alders have been reported from Great Britain, Ireland, Belgium, the Netherlands, Sweden, Denmark, France, Germany, Switzerland, Austria, Italy and Hungary (e.g. Anselmi et al. 2001, Brasier et al. 1995, Cech 2000, Osswald et al. 2001, Werres 1998). Some species from the genera *Phytophthora* and *Pythium* were found to be the cause of the disease. As the most harmful a new *Phytophthora* hybrid, so called "alder-*Phytophthora*" (Brasier 1995), was found.

In the Czech Republic an increasing decline of alders was noted at the end of the 1980s and at the beginning of the 1990s (Jančařík 1993). However, the possible cause of this disease, activity of pathogenic pythiaceous fungi in roots and trunks of trees, was not found.

During research by Gregorová et al. (2002) many reports of alder decline were obtained, mainly in river basins of the Labe and Ohře rivers. Several sites with

alder decline were visited in 2001 and 2002 and samples of soil with damaged roots and of necrotic trunk tissues were collected. One of the isolated fungi, the "alder-*Phytophthora*", was found at two localities by the Chodovský potok brook near Karlovy Vary. More than one hundred trees of different age have been killed at this locality in the past years and the stability of banks of the lower course of this brook disturbed.

MATERIALS AND METHODS

Study site

The strains of "alder-*Phytophthora*" were found at two localities near Karlovy Vary (district Karlovy Vary, north-west Bohemia). The first locality is the left bank of the Chodovský potok brook in Karlovy Vary-Dvory (50° 13' 42" N, 12° 49' 28" E) near the road to Počerny, the second one is the right bank of the Chodovský potok brook, about 150 m upstream of the bridge near Zátiší (50° 14' 14" N, 12°47' 20" E). This fungus was isolated from soil with damaged roots and from conductive trunk tissue of damaged trees of *Alnus glutinosa*.

Methods

The samples were processed the day after taking. The soil samples were cultivated by the baiting method, the samples of conductive tissue and bark were cultivated directly on agar media.

The soil samples with damaged roots were inserted into sterile glass containers and flooded by deionised sterile water. Young, surface-sterilised leaves of *Alnus glutinosa*, *Syringa vulgaris*, and *Rhododendron* sp. were floated over the water surface as baits. Samples were cultivated in the dark at 21 °C. After several days the baits were repeatedly investigated under a dissect microscope. Parts of leaves with characteristic coenocytic mycelium and/or zoosporangia or with expanding necrosis were cut and put on agar plates.

The samples of conductive tissues and bark of trunks were rinsed with sterile water and repeatedly shaken in sterile water with Tween 20. The samples were then cut into small fragments of tissue, which were rinsed again and placed on Petri dishes.

The water agar with benomyl (25 ppm), quintozene (100 ppm), and with penicillin (50 ppm) and the water agar without fungicides were used for isolation. The obtained strains were purified on the water agar medium and then cultivated and retained on oatmeal agar.

The isolated fungus was first identified as *P. cambivora* (Petri) Buisman according to Erwin and Ribeiro (1996). However, later it was shown that our

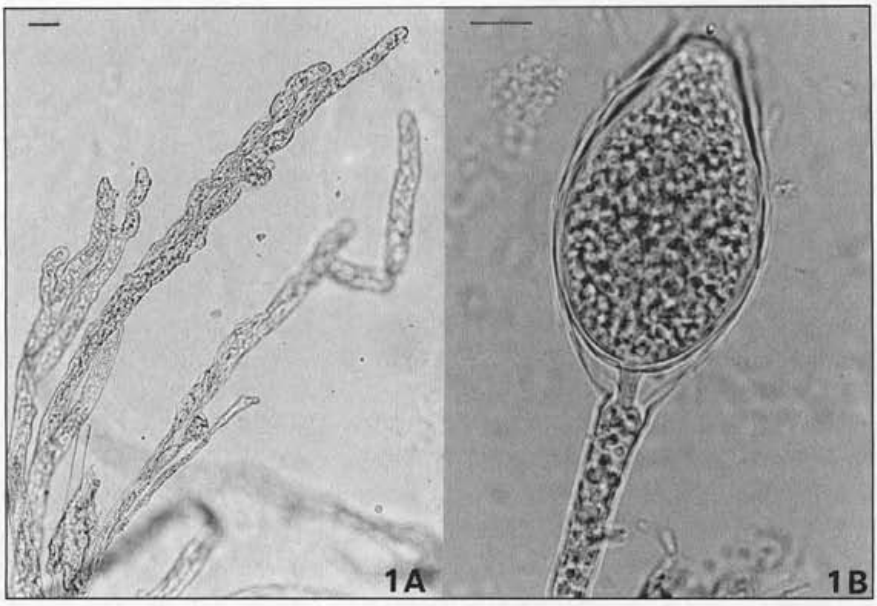

Fig. 1. "alder-*Phytophthora*". A: coenocytic hyphae, B: young nesting zoosporangium. Bars: 10 μm.

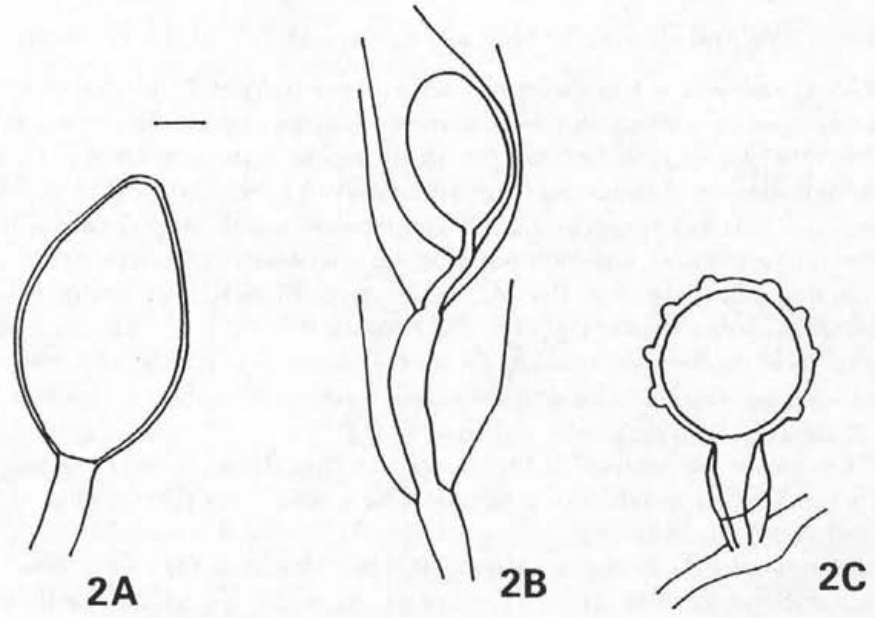

Fig. 2. "alder-*Phytophthora*". A: mature zoosporangium, B: proliferation of zoosporangiophores, C: oogonium and oospore. Bar: 10 μm.

isolates are very close to a fungus associated with alder mortality in Britain, described originally by Brasier et al. (1995). Strains are deposited at Agency for Nature Conservation and Landscape Protection of the Czech Republic.

RESULTS AND DISCUSSION

Frequency of fungus occurrence

The samples were taken throughout the year, but the main part of pythiaceous fungi were found only in summer and autumn. The samples from winter, spring and late autumn were often negative. Five strains of "alder-*Phytophthora*" were isolated in September 2001, the two other were acquired in July and October.

During research of the stands with alder-decline on the Chodovský potok brook several number of samples were taken. 34 samples of soil with roots (only 6 of them positive) and 12 samples of trunk necrosis (1 positive) were acquired. The low frequency of isolation (15.22 %) corresponds with the biology of parasitic pythiaceous fungi and with methodical difficulties of "alder-*Phytophthora*" isolation and corresponds to the obviously mentioned frequencies of isolation (e.g. Jung 1998).

Description and distinguishing characters of "alder-*Phytophthora*"

Colony diameter 6–8 cm/week on oatmeal agar (OA) at 21 °C; the colony is appressed and has sometimes sparse aerial mycelium; mycelium hyaline, coenocytic (older mycelium may be septate), branched, hyphae with diameter 6.6–9.8 μm with numerous small inclusions (Fig. 1A); coralloid hyphae (typical of *P. cambivora*) and chlamydospores absent. Sporangiophores mainly simple, unbranched. Zoosporangia terminal, non-caducous, ellipsoid, sometimes ovoid, non-papillate or with a minute papilla (Fig. 1B, 2A); internally proliferate, often nested within the original zoosporangium (Fig. 1B, 2B); measuring 49–62 × 28–32 μm. Oogonia sparse, their numbers diminishing during the time of cultivation and many of them aborting. Oogonia terminal, sphaerical, thick-walled, tuberculate, diameter 30–44 μm (Fig. 2C), antheridia amphigynous.

This fungus was described in 1995 for the first time (Brasier et al. 1995) and its taxonomic position was discussed. The following genetic study (Brasier et al. 1999) showed that "alder-*Phytophthora*" is not a single taxonomic entity but an array of phenotypically highly diverse heteroploid genotypes, thus the fungus does not have a scientific name at present. The first parent of this fungus is *P. cambivora*, the second one is still unknown, but it is close to *P. fragariae*. Both fungi are thought to be introduced in Europe (Brasier et al. 1999).

The pathogen differs from its parents by pathogenicity; *P. cambivora* and *P. fragariae* do not have the capacity to cause disease in alder (Brasier et al. 1999). The growth optimum of pathogen is lower than the growth optimum of *P. cambivora* (Brasier et al. 1995).

The isolated hybrid "alder-*Phytophthora*" is morphologically very close to its parent species, especially *P. cambivora*. The isolated fungus differs by its self-fertility (its parents are heterothallic), but the production of oogonia may be partially disrupted in many strains (Brasier et al. 1995). The other morphologic characters are similar to the characters of *P. cambivora*. The pathogenic strains are partially different by the felty appearance of their colonies and the sparse aerial mycelium, and by the presence of some smaller oogonia.

Symptoms and disease development

The first symptoms are the presence of small, chlorotic leaves, dying leaves and the appearing of leafless twigs in the crown of alders during summer. The foliage may be sparse and faded in one part or in the entire crown, especially when the disease is acute. Reddish-brown or brown spots and necrosis on bark appear on the basis of stem. Under these spots strips of necrotised conductive tissue projecting from the base of the tree develop. When most tissue around the trunk is killed by the pathogen, the foliage become sparse, the dying of branches is progressive; in a later stage of the disease the tree has a skeletal appearance. Sometimes a secondary overgrow develops. The root system and conductive tissue of these trees are highly reduced and the disease symptoms may look like tracheomycosis.

The symptoms of decline are similar in the whole area of alder decline in Europe, but the impact of the fungus on native alder populations is greatly varying in dependence of race of pathogen and conditions of the stand. The standard variant of "alder-*Phytophthora*" is more damaging to alder than some other variants, e.g. the "Swedish variant" in northern Europe (see Brasier et al. 1999, Werres 1998).

There are some other species of *Phytophthora* which are probably involved in the disease. These include *P. gonapodyides*, *P. citricola*, and *P. syringae* (Jung et al. 2000, Osswald et al. 2001). We found *P. syringae* associated with the alder decline at one other locality near Karlovy Vary.

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